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Auxin-regulated Wall Loosening and Sustained Growth in Elongation[1]

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LARRY N. VANDERHOEF[2] AND ROLAND R. DUTE[3]
Department of Botany, University of Illinois, Urbana, Illinois 61801

ABSTRACT

It is proposed that auxin regulates and coordinates both wall loosening and the supply of wall materials in elongation. The tenets of the proposal allowed testable predictions. It was determined that, if the cell walls of *Glycine max* L. var. Wayne hypocotyl segments are maintained in a loosened state (by excising the segments directly into pH 4 medium), exogenous auxin induced only the second response. It was also predicted and confirmed that elongating systems, *e.g.* pea epicotyl, with certain early auxin-induced growth kinetics (an initial high non-steady-state rate followed immediately by a drop to a lower steady-state rate) would show a transient second response (in addition to the usual transient first response) when stimulated by pH 4 medium. Finally, it is pointed out that recent results which establish the existence of auxin-induced elongation-associated proteins support the proposition that auxin coordinates wall loosening and the supply of wall materials in elongation.

The mode of action of auxin-regulated elongation is not known. Recently, two hypotheses have guided research in this area. In the 1960s, the gene expression hypothesis proposed that auxin regulated wall loosening and steady-state elongation by action at gene transcription or translation (9, 14). However, recognition of the short lag between auxin application and detectable elongation rate increase (10, 12, 25) by Evans and Ray (6) was thought to rule out primarily mediation at gene expression in auxin-regulated wall loosening (11). In 1971, the wall-acidification hypothesis (4, 13) was independently proposed by Hager *et al.* (7) and Cleland (3). This hypothesis, which proposes that auxin regulates wall loosening by causing a pH drop in the area of the cell wall, has survived a series of rigorous challenges (4). The molecular mechanism of auxin-caused wall acidification remains to be described.

In 1975, we commented on the unusual early kinetics which are seen when auxin stimulates elongation in auxin-depleted (hence, slowly growing), excised elongating segments (20). We suggested, and others have confirmed (8), that auxin-induced elongation could be separated into two phases, the early burst of growth (simulated by lowering the pH from 6 to 4) and a later phase associated with long-term, steady-state growth. Subsequent experiments showed that elongation during the first phase was biochemically distinct from elongation during the second phase (15, 16, 21, 23).

Experiments reported herein support the proposal that auxin coordinates wall loosening and the supply of wall materials in its regulation of cell elongation.

MATERIALS AND METHODS

Soybean seedlings (*Glycine max* L. Merr. var. Wayne) were germinated in the dark for 3 days, and the elongating segment of the hypocotyl was excised as described (20, 22). Pea seedlings, *Pisum sativum* var. Alaska, were germinated in the dark in moist vermiculite at 25 C for 4 days. Elongating segments were excised from the first internode of the epicotyl immediately below the hook. Hypocotyl and epicotyl extension were measured continuously with a linear position-sensitive transducer in an apparatus modified after that reported (19), except that the clamped segments were immersed in 25 C medium. The pH 6 medium, 5 mM K-phosphate, and the pH 4 medium, 5 mM K-citrate, contained 30 mM sucrose. IAA was added to a final concentration of 45 μM in the soybean experiments.

RESULTS AND DISCUSSION

The research findings described in the introduction agree with and support the hypothetical scheme of cell elongation shown in Figure 1. The figure is based on the data that prove the existence of separable responses, on the data which support the wall-acidification and gene expression hypotheses and on the consideration of what very likely happens to an excised rapidly elongating segment which is preincubated and then treated with acid or auxin. The figure depicts two auxin activities in the control of growth, wall loosening and the regulation of the supply of wall materials. During steady-state growth in the intact plant, these two activities would be occurring simultaneously and would be indistinguishable in measurements of elongation. But when the elongating segment is excised, preincubated in the absence of auxin, then reintroduced to auxin, the two events are not reinitiated simultaneously, and they can be distinguished from each other. The loosening event occurs first, resulting in a burst of growth (the "first response"). Then wall material synthesis resumes, resulting in the steady-state growth of the "second response."

This suggested sequence of events in the excised segment allows a testable prediction: if the wall is kept loose from the moment of excision and throughout the preincubation (during auxin depletion), then reinitiation of elongation by exogenous auxin should occur in the absence of the short-lag burst of growth which occurs during wall loosening. That is, exogenous auxin should induce just the second response. Since it has previously been shown (17) that acid-induced growth mimics the auxin-induced first response (wall loosening), the experiment was feasible. Segments were excised from the rapidly elongating region of the soybean hypocotyl and mounted directly into the growth-measuring apparatus. The segments were held in the apparatus at 10 g tension with the

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[2] Present address: Division of Agricultural and Life Sciences, University of Maryland, College Park, MD 20742.

[3] Present address: Department of Biology, St. Ambrose College, Davenport, IA 52803

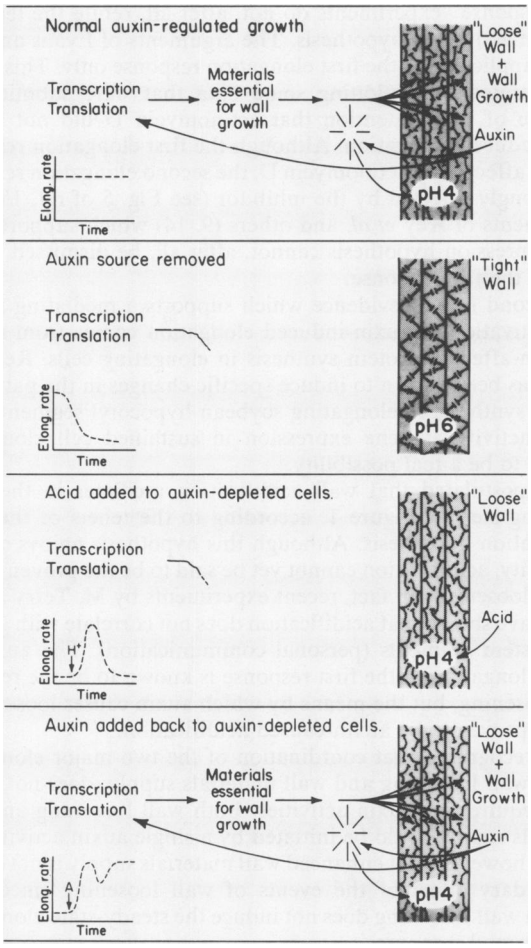

FIG. 1. Events in cell elongation. Auxin is postulated to regulate and coordinate both wall loosening and wall synthesis. Top panel, elongation in the intact seedling. A continuous supply of auxin keeps the wall loose by maintaining a low wall pH, and the cells growing by maintaining the supply of material(s) essential for wall growth. Thus, there is a steady-state growth rate. Second panel, growth in an excised elongating segment. During the first 30 to 90 min after excision, elongation decreases to a low rate in the absence of endogenous auxin. The wall is not maintained in a loosened state; the supply of growth-essential materials is terminated. Third panel, acid-induced growth in auxin-depleted, excised segments. Acid mimics the wall-loosening component of auxin-regulated elongation, causing a burst of growth. Thus, acid does not induce a steady-state elongation rate. Rather, the rate rises with almost no lag after acid addition and then begins to decline. Bottom panel, auxin-induced growth in auxin-depleted, excised segments. The first observable effect of added auxin is the burst of growth caused by wall loosening. The rate rises, then begins to fall, with kinetics very similar to acid-induced growth. However, the auxin-caused insertion of wall materials begins about 50 min after auxin addition, and the rate rises once again, eventually reaching a steady-state rate. Thus, the two auxin-regulated components of elongation can be individually observed only when exogenous auxin is added to auxin-depleted segments. Their separation occurs because the lag times for the two components are different, *i.e.* auxin-regulated wall acidification occurs with a lag near 15 min, whereas auxin-regulated wall materials supply begins with a lag near 50 min.

medium buffered at pH 4. In the absence of endogenous auxin, the growth rate of the segment decreased with time from the rate in the intact seedling (about 0.7 mm h^{-1}). After 50 min, when the elongation rate was near 0.2 mm h^{-1}, exogenous auxin was added to the medium. This experiment, with appropriate controls (no auxin, pH 6), is shown in Figure 2. As predicted, when the wall was kept loose at pH 4, exogenous auxin induced a normal second response, but the first response was significantly diminished. The elimination of the first response when the segments are preincubated in pH 4 buffer was absolutely dependent on buffer strength. The results of Figure 2 were obtained with 5 mM buffer, but not with 1 mM buffer. The higher buffer strength was required because of the strong buffering capacity of the wall at pH 5.5. We demonstrated this buffering capacity in 1977 (see Fig. 1 of ref. 17), and in 1979 Cleland pointed out its possible role in masking auxin-induced medium acidification by segments (4). Past ambiguous or inconsistent results (*e.g.* see Fig. 4 and "note added in proof" of ref. 18) were likely caused by wall buffers.

The "missing growth" of the first response at pH 4 does not show up as additional growth between 0 time and the time of auxin addition. However, such additional growth would not be expected if the limiting factor during these first 40 min of declining growth rate was (*a*) something other than wall-tightening, and (*b*) the same at both pH 4 and pH 6. Such a common limiting factor is entirely conceivable.

Events of auxin-induced elongation, as depicted (Fig. 1), allowed another prediction: there should be circumstances where acid-induced growth (in excised, auxin-depleted segments) will mimic not just the first response, but a transient second response as well. This would occur where some of the wall materials (either already made or destined to be made "in the pipe" at the time of excision) do not get into the wall during the postexcision preincubation. Examination of the kinetics of several species (2, 23) suggested that pea epicotyl should show this phenomenon. This could be predicted because of the type of elongation kinetics which occur when exogenous auxin is added to auxin-depleted elongating pea epicotyl segments. Figure 3 shows these kinetics, replotted from Figure 1 of reference 2. This type of kinetics results when the lag time for the second response is unusually short (see discussion of Fig. 5 in ref. 20). This shortened lag period for the second response, in turn, would result if there was a pool of unused wall materials in the auxin-depleted excised segment at the time of auxin addition.

The auxin-induced elongation kinetics shown (Fig. 3) allows the prediction that acid-induced elongation will show a transient second response, in addition to the expected first wall-loosening response. The experiment gave the predicted result (Fig. 4); a

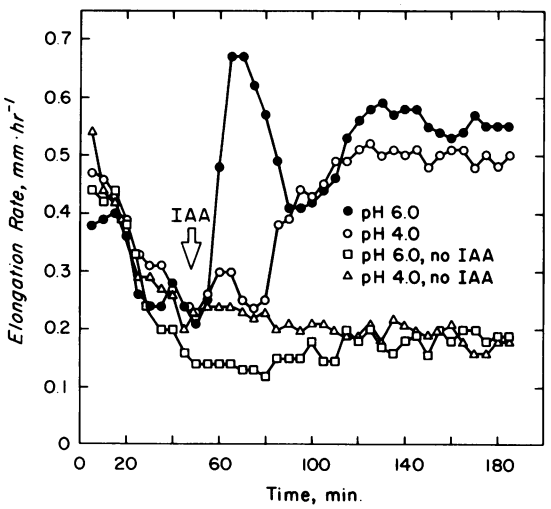

FIG. 2. Auxin induces only the second response in acid-pretreated soybean segments. The excised elongating segment was transferred immediately to pH 4 or 6 medium in the growth-measuring apparatus. Auxin (final concentration, 45 μM) was added when the elongation rate decreased to near 0.2 mm·h^{-1}.

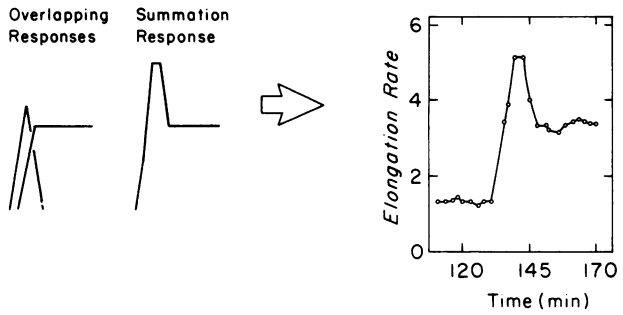

FIG. 3. Auxin-stimulated elongation kinetics in auxin-depleted pea segments. Replotted from Figure 1B of reference 2.

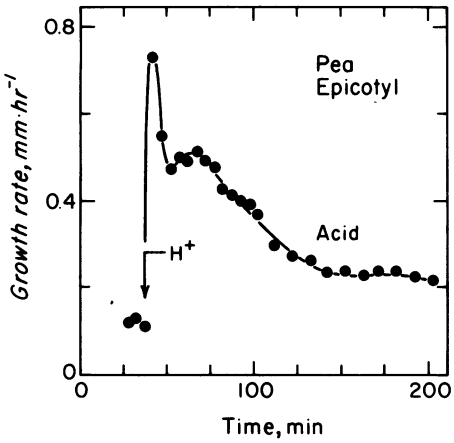

FIG. 4. Acid-stimulated growth in pea epicotyl segments. Segments were preincubated at pH 6.0 until the elongation rate dropped to 0.2 mm h^{-1} (about 1.0 h). The medium then was replaced with pH 4 K-citrate at the arrow.

preliminary report of this result has appeared (5) simultaneously with an independent verification of the predicted result (1).

It has recently been determined (R. R. Dute, unpublished data) that the results of soybean hypocotyl experiments described herein and previously are quite repeatable for other stem-like tissues (pea epicotyl, corn mesocotyl). However, this is not always true for the leaf-derived coleoptile. For example, although acid-induced growth is transient in the coleoptile (evidence that acid-induced growth and the first response are equivalent), one cannot separate the two responses by use of cytokinin.

CONCLUSIONS

The gene expression and wall-acidification hypotheses are not incompatible. All available data can be accommodated if one assumes that auxin regulates and coordinates both wall loosening and the supply of wall materials (Fig. 2). Thus, the reintroduction of auxin to excised, auxin-depleted segments causes wall loosening (perhaps mediated by wall acidification) and a subsequent burst of turgor-driven elongation. This elongation is transient; it lasts 30 to 90 min, depending on conditions and species. Soybean hypocotyl elongation cannot continue by this first wall-loosening auxin action alone. This very important point was first established with experiments which employed cytokinin, an elongation inhibitor (20, 22). Auxin must have another activity in its regulation of cell elongation, very likely the regulation of the production of compounds necessary for sustained cell elongation.

There is little direct evidence for gene expression regulation by hormones in a eucaryotic system. However, this kind of auxin action must be considered most likely, in light of several lines of indirect and circumstantial evidence. First, it is now clear that the "fast response" experiments do not, after all, refute the tenets of the gene activation hypothesis. The arguments of Evans and Ray (6) are applicable to the first elongation response only. This is best demonstrated by replotting some data that were submitted in evidence of the contention that actinomycin D did not inhibit auxin-induced elongation. Although the first elongation response was not affected by actinomycin D, the second elongation response was strongly affected by the inhibitor (see Fig. 5 of ref. 15). The experiments of Key *et al.* and others (9, 14) which supported the gene expression hypothesis cannot, after all, be dismissed on the basis of the fast response.

A second line of evidence which supports a mediating role of gene activation in auxin-induced elongation comes from studies of auxin-affected protein synthesis in elongating cells. Recently, auxin has been shown to induce specific changes in the pattern of protein synthesis in elongating soybean hypocotyl segments (26). Auxin activity at gene expression in sustained cell elongation appears to be a real possibility.

It is postulated that wall acidification mediates in the wall-loosening step of Figure 1, according to the tenets of the wall-acidification hypothesis. Although this hypothesis enjoys current popularity, acidification cannot yet be said to be the proven means of wall loosening. In fact, recent experiments by M. Terry and R. Jones have shown that acidification does not correlate with growth in pea stem segments (personal communication). The auxin-induced elongation of the first response is known to be the result of wall loosening, but the means by which auxin causes loosening is not yet proven to be auxin-caused acidification.

It is recognized that coordination of the two major elongation events, wall loosening and wall materials supply, does not necessarily require two auxin activities. Both wall loosening and wall materials supply could be initiated by a single auxin activity. It is known, however, that enhanced wall materials supply is not simply a secondary effect of the events of wall loosening since acid-induced wall loosening does not induce the steady-state elongation of the second response.

A final point can be made regarding the hypothesis of Figure 1. The unusual early elongation kinetics led first to the postulate that there were separable responses (20), then to the conclusion that elongation during the early phase of elongation (in auxin-stimulated elongation in excised segments) was biochemically distinct from elongation during the later phase (8, 21, 23), and finally to an explanation of the two phases (this manuscript and refs. 15 and 16). The hypothesis is quite specific in omitting any consideration of osmoregulation. The experiments from Boyer's laboratory (24) have made it clear, after years of debate, that auxin-induced changes in cell enlargement cannot be attributed to changes in cell osmotic potentials or turgor.

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